Published in Histochemie 25: 247-255, 1971

AFRRI SR70-6
JUNE 1970

AFRRI
SCIENTIFIC
REPORT

A HISTOCHEMICAL STUDY OF LIVER ENZYMES INVOLVED IN GLYCOGEN METABOLISM IN THE X IRRADIATED RAT

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Atomic Support Agency
Bethesda, Maryland

AFRRI SR70-6

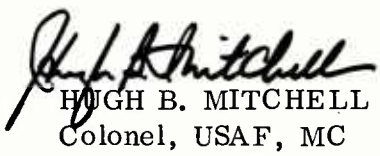
All aspects of investigative programs involving the use of laboratory animals sponsored by DoD components are conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care", prepared by the National Academy of Sciences - National Research Council.

AFRRI SR70-6
June 1970

A HISTOCHEMICAL STUDY OF LIVER ENZYMES INVOLVED IN GLYCOGEN METABOLISM IN THE X IRRADIATED RAT

G. N. CATRAVAS
C. G. McHALE

S. J. BAUM
Chairman
Experimental Pathology Department



HUGH B. MITCHELL
Colonel, USAF, MC
Director

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Atomic Support Agency
Bethesda, Maryland

ACKNOWLEDGMENT

The authors wish to thank O. Z. Williams for his capable technical assistance during the course of this study. They also wish to express their gratitude to J. T. Blake for his invaluable help in electron microscopy.

TABLE OF CONTENTS

LIST OF FIGURES

FOREWORD
(Nontechnical summary)

Glycogen, sometimes called "animal starch", is a highly branched macromolecule composed essentially of glucose units. It is the main carbohydrate storage material in mammalian systems and constitutes the most important store of energy capable of rapid mobilization. Liver glycogen is reversibly convertible to blood glucose and serves to maintain the blood sugar level when the supply of sugars through intestinal absorption is not adequate.

In vivo, liver glycogen is converted to glucose by means of the enzymes amylophosphorylase and amylo-1,6-glucosidase (debranching enzyme). Its synthesis from glucose is catalyzed by the enzymes UDPG transglucosylase and 1,4 → 1,6 transglucosidase (branching enzyme).

Several investigators have reported that the glycogen content of the liver of rats and other animals increases after irradiation. It was therefore of interest to determine if and to what extent radiation affects the activities of the above enzymes. This report describes a cytochemical study of the changes which occur in the activity of these enzymes following whole-body x irradiation of rats. It was found that cytochemically demonstrable changes do occur and that the activities of the enzymes responsible for glycogen breakdown to glucose are markedly reduced by radiation. The same was found for 1,4 → 1,6 transglucosidase (branching enzyme). In contrast, the activity of UDPG transglucosylase which is responsible for the in vivo synthesis of 1,4-polysaccharides was found to be enhanced by x irradiation of the animal.

ABSTRACT

The liver enzymes responsible for the breakdown and synthesis of glycogen from glucose have been investigated cytochemically in rats exposed to 1200 rads of x rays. It was found that significant changes occur in their activities and that amylophosphorylase and amylo-1,6-glucosidase, both of which are responsible for the conversion of glycogen to glucose, are markedly inhibited by radiation. A significant inhibition of the activity of 1,4 →1,6 transglucosidase (branching enzyme) was also observed. In contrast, the activity of UDPG-glycogen transglucosylase, which is responsible for the in vivo synthesis of 1,4-polysaccharides, was found to be enhanced.

I. INTRODUCTION

Several investigators have reported that the glycogen content of the liver of rats and also of mice increased after exposure of the animals to x rays.[5,6,16] Radiation-induced glycogen changes have also been demonstrated cytochemically in liver and brain preparations from irradiated rats.[4,8-10,13] Using biochemical methods we have previously shown that glycogen phosphorylase and amylo-1,6-glucosidase both of which are involved in the breakdown of glycogen to glucose are greatly inhibited by irradiation of the animal.[1] A considerable inhibition in the activity of amylo-(1,4 → 1,6)-transglucosidase was also observed. In contrast, the activity of UDPG transglucosylase which is responsible for the in vivo synthesis of 1,4-polysaccharides was found to be greatly enhanced by radiation.[1] The present study deals with the cytochemical demonstration of radiation-induced changes of the above-mentioned enzymes.

II. PROCEDURES

Female Sprague-Dawley rats of the Charles River strain weighing 90 to 110 grams were used in this study. All animals were kept on a fat-free diet at least 7 days prior to irradiation. Each animal received approximately 12-13 grams of food (dry weight) per day. The entire ration was given at one time and the rats were trained to consume it within 1 to 1-1/2 hours. Radiation exposures started approximately 2 hours after the animals had consumed their food. No food was given to the animals following irradiation. Control rats were also deprived of food over periods of time corresponding to those for the irradiated rats. All animals had free access to water.

This study used a total of 88 rats in 11 experiments. The animals utilized in each experiment were divided into two equal groups, i.e., four rats were irradiated and the remaining four served as unirradiated controls. The experimental animals were exposed to 1200 rads of whole-body x rays. During exposure the animals were individually housed in Lucite boxes which were so arranged in the radiation field that each rat received an equal unilateral exposure. The tissue dose rate at the midline of the animal was 20 rads/min in air. The distance from the x-ray tube to the midline of each rat was 100 cm. The physical characteristics of the exposure source were as follows: 250 kVp, 30 mA, 1.2 mm beryllium and added filtration of 0.95 mm copper (HVL-1.9 mm Cu).

Following irradiation two animals from each experimental group as well as from the unirradiated controls were sacrificed at 24 or 48 hours postirradiation. The livers were quickly excised and pieces approximately 5 x 8 x 3 mm of the left lateral lobe of the liver were removed. These pieces were frozen at -20°C within 1/2 minute after decapitation. When samples for electron microscopy were desired, liver specimens were rapidly cut in approximately 1 mm cubes and placed immediately in fresh 4 percent glutaraldehyde buffered with 0.1 M phosphate buffer at pH 7.2.

Cytochemistry. A sliding microtome mounted in a cryostat (International Equipment Co.) maintained at -20°C was used to prepare frozen liver sections 15 μm thick which were then mounted on microscope slides. The slides with the adhering tissue sections were stored in the cryostat (usually less than 10 minutes) until ready for incubation. Plastic cylindrical vials with raised side ridges to hold the microscope slides (Scientific Products) were utilized as incubation containers. Incubations

were carried out for 2 hours at 37°C with gentle shaking. Duplicate specimens were used.

Phosphorylase. Phosphorylase activity was demonstrated in the direction of synthesis of polysaccharide from glucose. Takeuchi and Kuriaki's method[20] with minor modifications was used. The incubation medium (10 ml) contained 0.6 mM glucose-1-phosphate (K salt); 3 mM adenosine-5'-phosphate; 0.4 mM NaF; 40 μM $MgSO_4$; 1.5 μM $HgCl_2$; 3 mg glycogen; and 0.8 mM acetate buffer pH 5.7. Following incubation the slides with the mounted liver sections were rinsed with 40 percent ethanol, followed by water. They were then dried at room temperature, fixed for 3 minutes in absolute ethanol and dried again at room temperature. Staining was carried out by immersing the mounted liver sections for 3 minutes in Gram's iodine solution ($I:KI:H_2O$ 1:2:900).

1,4 → 1,6 Transglucosidase. The incubation medium was the same as for phosphorylase except that no $HgCl_2$ was added. Following incubation the liver sections were treated for 1 minute at 37°C with an aqueous solution of β-amylase (0.25 percent) buffered to pH 5.7 with acetate buffer (1 part 0.5 M buffer per 100 ml). Staining was performed as described earlier.

UDPG Transglucosylase. Takeuchi and Glenner's method[19] was employed to demonstrate UDPG transglucosylase activity.

1,6 Glucosidase. The incubation medium (10 ml) contained 1 mM potassium phosphate pH 6.8 and 0.05 ml of α-phosphorylase suspension in 0.0015 M EDTA, 0.1 M NaF, 0.00192 M sodium glycerophosphate (pH 6.8; 15.5 mg protein/ml; 1750 units/mg; Sigma Co.), which was added just before starting the incubation. The

mounted liver sections were incubated for 5 minutes at 30°C. They were then fixed and stained as described earlier.

Electron microscopy. The liver specimens were left in the glutaraldehyde fixative overnight. They were then washed in a solution of 0.05 M cacodylate buffer pH 7.2 and postfixed in 1 percent osmium tetroxide.[11] Following fixation the tissues were dehydrated in graded ethanol solutions and embedded in Maraglas.[15] The blocks were cut with a Porter-Blum MT2 ultramicrotome and sections mounted on uncoated grids. After staining with uranyl acetate[14] and lead citrate[21] the sections were examined in the electron microscope (Siemens Elmiskop 1A).

III. RESULTS

Figure 1 shows an electron micrograph of a liver preparation obtained from a fed unirradiated rat and Figure 2 from a rat starved for 24 hours. It can be seen in these figures that the livers of fed animals contain large deposits of glycogen. In contrast, those of starved rats exhibit only small quantities of glycogen since, as expected, their stores were depleted by starvation. Electron micrographs of liver preparations from x irradiated rats 24 and 48 hours after exposure are illustrated in Figures 3 and 4, respectively. As shown in these figures, large deposits of glycogen are still present although the animals did not receive any food after irradiation.

Figure 5 shows a liver section obtained from a control (unirradiated) rat and incubated in the phosphorylase medium. It exhibits large deposits of polysaccharide synthesized by the action of phosphorylase. That this polysaccharide was actually synthesized and not already present is illustrated in Figure 6 which shows a similar liver section incubated in the same medium but without glucose-1-phosphate and

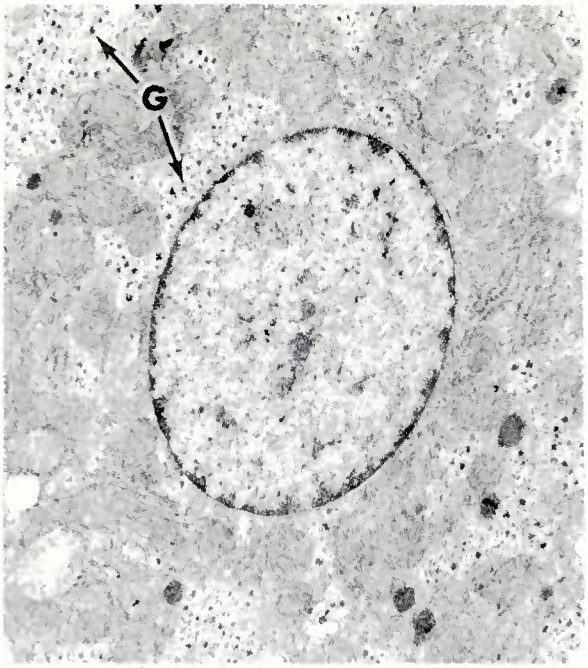


Figure 1. Liver cell of a fed unirradiated rat.
X 8,000 G = glycogen

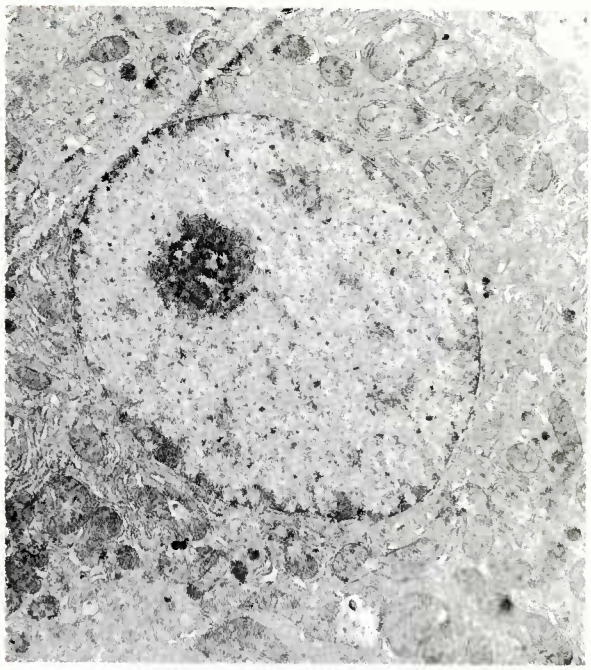


Figure 2. Liver cell of an unirradiated rat starved for 24 hours. X 10,000

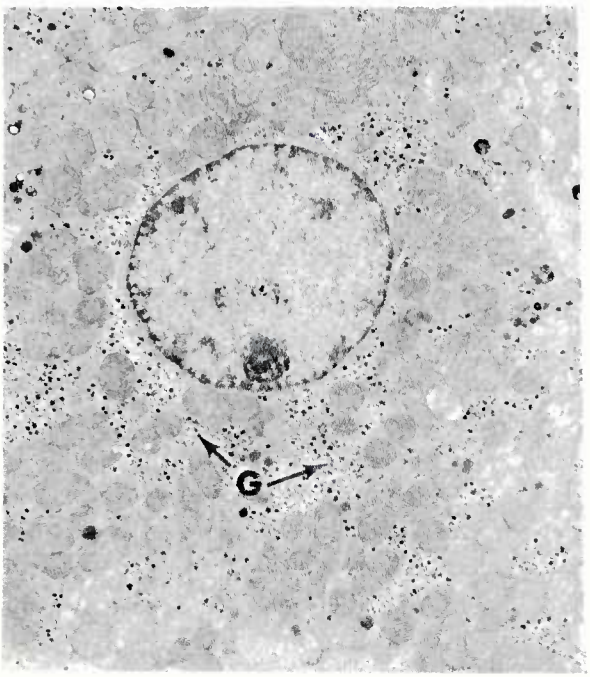


Figure 3. Liver cell of an irradiated rat 24 hours after irradiation.
X 4,800 G = glycogen

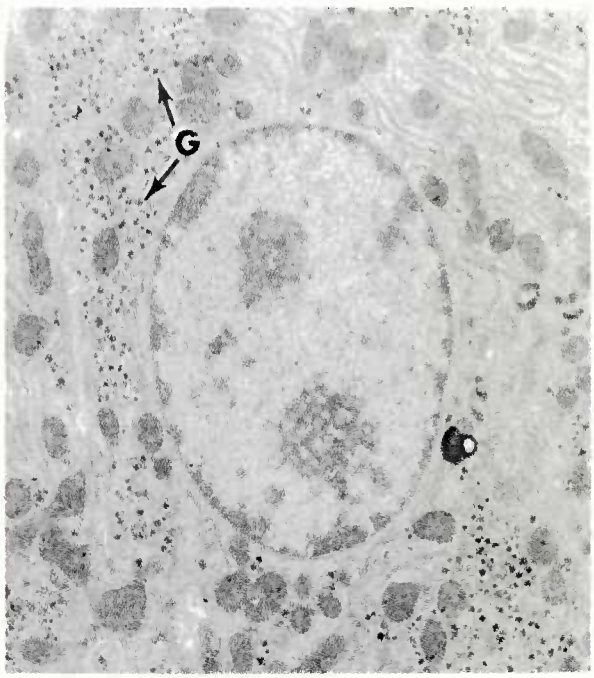


Figure 4. Liver cell of an irradiated rat 48 hours after irradiation.
X 9,000 G = glycogen

adenosine-5'-phosphate. No deposits of polysaccharide can be detected. A liver section obtained from a control (unirradiated) rat and incubated in the 1,4 → 1,6 transglucosidase medium is shown in Figure 7. The presence of polysaccharide deposits indicates significant transglucosidase activity.

The amounts of polysaccharide synthesized by the above two enzymes in liver sections from rats exposed to 1200 rads of x rays and sacrificed 24 hours later are illustrated in Figures 8 and 9. It can be seen in these figures that only small amounts of polysaccharide are present which indicates that the activities of both enzymes, i.e., phosphorylase and 1,4 → 1,6 transglucosidase are markedly reduced by irradiation of the animal. UDPG transglucosylase activities in the livers of control (unirradiated) rats and animals exposed to x rays and sacrificed 24 hours after exposure are shown in Figures 10 and 11, respectively. As shown in these two figures the amounts of polysaccharide synthesized indicate that the activity of this enzyme is stimulated by x irradiation.

Figure 12 illustrates a liver section obtained from an unirradiated fed rat and stained in Gram's iodine solution. Figure 13 shows a similar liver section which, prior to fixing and staining, was incubated in the 1,6 glucosidase medium. It can be seen that the amount of unhydrolyzed glycogen is greatly reduced indicating significant 1,6 glucosidase activity. Figure 14 shows a liver section obtained from an irradiated rat sacrificed 24 hours after exposure and incubated in the 1,6 glucosidase medium. It is obvious that the amount of unhydrolyzed glycogen is larger than that in the liver section from the unirradiated control (Figure 13). This indicates that the activity of this debranching enzyme has been impaired by radiation. Results similar

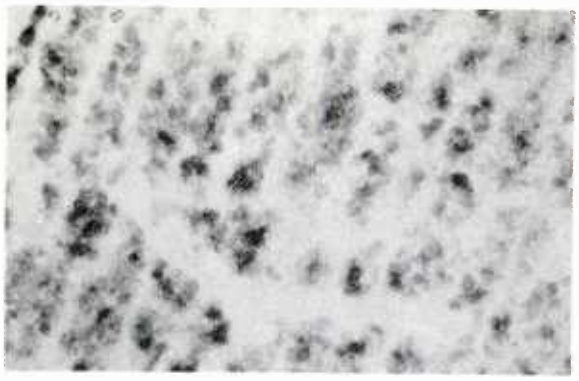


Figure 5. Liver section obtained from an unirradiated rat and incubated in the phosphorylase medium. (Stained in Gram's solution.)

Figure 6. Liver section obtained from an unirradiated rat and incubated in the phosphorylase medium without AMP and glucose-1-P. (Stained in Gram's solution.)

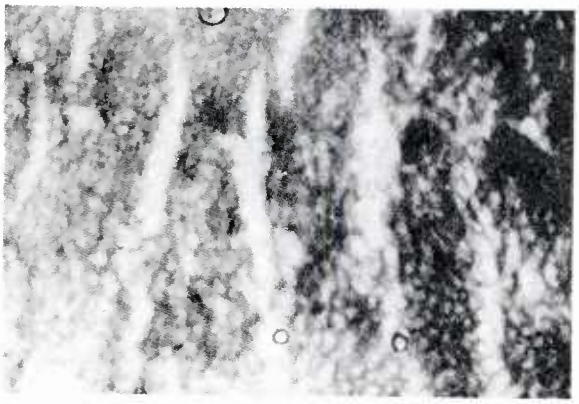


Figure 7. Liver section obtained from an unirradiated rat and incubated in the 1,4 → 1,6 transglucosidase medium. (Stained in Gram's solution.)

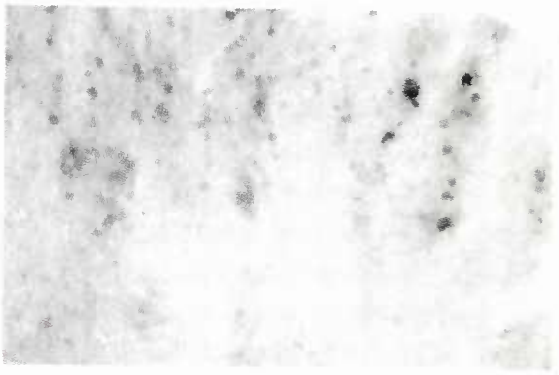


Figure 8. Liver section obtained from an irradiated rat (24 hours postirradiation) and incubated in the phosphorylase medium. (Stained in Gram's solution.)

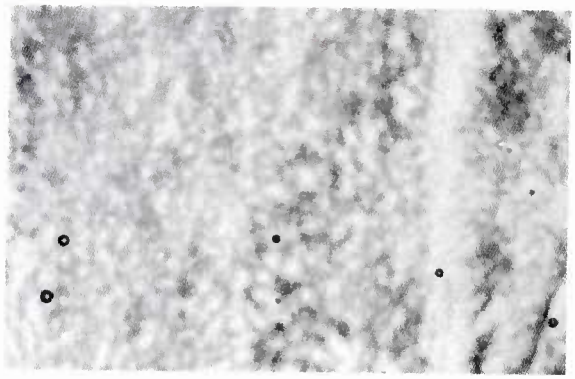


Figure 9. Liver section obtained from an irradiated rat (24 hours postirradiation) and incubated in the 1,4 → 1,6 transglucosidase medium. (Stained in Gram's solution.)

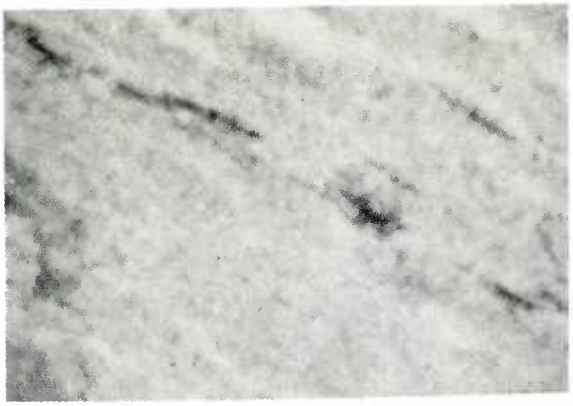


Figure 10. Liver section obtained from an unirradiated rat and incubated in the UDPG transglucosylase medium. (Stained in Gram's solution.)

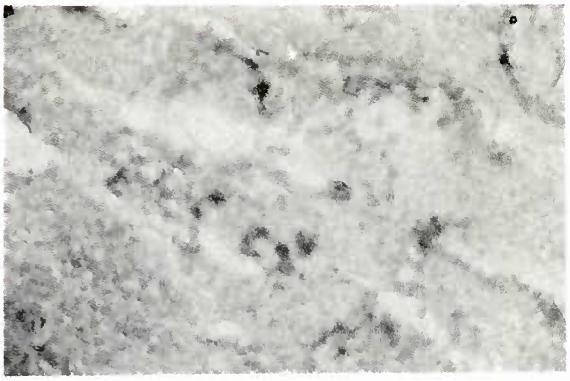


Figure 11. Liver section obtained from an irradiated rat (24 hours postirradiation) and incubated in the UDPG transglucosylase medium. (Stained in Gram's solution.)

Figure 12. Liver section obtained from an unirradiated fed rat and stained in Gram's iodine solution.

Figure 13. Liver section obtained from an unirradiated fed rat and incubated in the 1,6 glucosidase medium. (Stained in Gram's solution.)

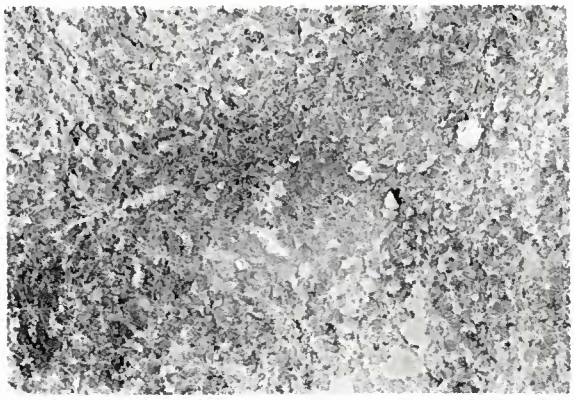


Figure 14. Liver section obtained from an irradiated rat (24 hours postirradiation) and incubated in the 1,6 glucosidase medium. (Stained in Gram's solution.)

to those described above for rats sacrificed at 24 hours were obtained for animals sacrificed at 48 hours postirradiation.

IV. DISCUSSION

It is known that the glycogen content of liver and other tissues is affected by the dietary condition of the animal and that starvation results in depletion of the glycogen stores. Electron micrographs taken of liver preparations from fed and starved rats (Figures 1 and 2) verify this effect. It has been shown also that, as seen in the electron microscope, the deposits of glycogen in the liver do not decrease following irradiation of the animal although no food was available between exposure and sacrifice (Figures 3 and 4). To clarify these observations a cytochemical study of the activity changes of enzymes responsible for the breakdown and synthesis of glycogen from glucose was undertaken. It was found that under our experimental conditions the activities of the enzymes amylophosphorylase and 1,6 glucosidase are markedly decreased by x irradiation of the animal. Both these enzymes are involved in the breakdown of glycogen to glucose units. It was also found that the activity of 1,4 → 1,6 transglucosidase (branching enzyme) is impaired by radiation. In contrast, the activity of UDPG transglucosylase which is responsible for the synthesis of 1,4-polysaccharides[7] is stimulated by irradiation of the animal.

To avoid the possibility of reactivating phosphorylase previously inactivated by irradiation and therefore interfering with the effect of radiation, cyclic-3',5'-AMP, a cofactor which mediates the conversion of inactive phosphorylase b to its active form a,[3] was omitted from the incubation medium when phosphorylase activity was to be determined. For the same reason glucagon or epinephrine was not added to the

incubation medium since these hormones act by enhancing the production of cyclic-3',5'-AMP.[2,12,17] The omission of these compounds, in all probability, would displace the corresponding enzymic reactions from their optimal conditions. However the differences in the amounts of polysaccharide synthesized were distinct enough to show clearly the effect of radiation on the enzymes under study.

To demonstrate phosphorylase activity in the presence of 1,4 → 1,6 transglucosidase, $HgCl_2$ was added to the incubation medium since this compound is known to inhibit the activity of the latter enzyme.[18]

As mentioned earlier, rats sacrificed at 48 hours after irradiation were also studied in these experiments. However, only the results obtained with liver sections from animals sacrificed 24 hours after exposure have been presented here since no detectable differences were observed between these two sets of animals. These results indicate that, under our experimental conditions, glycogen breakdown is markedly inhibited by irradiation of the animal, whereas the rate of synthesis of polysaccharide is enhanced. On the basis of these findings the accumulation of glycogen, which has been observed to occur in the liver of the irradiated animal and which has been verified by the electron micrographs shown, could be easily explained.

REFERENCES

1. Catravas, G. N. and McHale, C. G. Activity of rat liver enzymes responsible for glycogen metabolism after whole-body irradiation. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR70-3, 1970.

2. Exton, J. H. and Park, C. R. Stimulation of gluconeogenesis from lactate by epinephrine, glucagon, and cyclic 3',5'-adenylate in the perfused rat liver. Pharmacol. Rev. 18:181-188, 1966.

3. Grillo, T. A. I. A histochemical study of phosphorylase in the tissues of the chick embryo. J. Histochem. Cytochem. 9:386-391, 1961.

4. Gubin, G. D. Histochemical studies of ionizing radiation effects on nucleic acids and glycogen dynamics in rat livers. Radiobiologiya 2:553-557, 1962.

5. Hansen, L. The increase in liver glycogen in non-fasted rats after irradiation. A comparison with pair-fed animals. Int. J. Radiation Biol. 12:367-372, 1967.

6. Kay, R. E. and Entenman, C. Hyperglycemia and increased liver glycogen in rats after x-irradiation. Proc. Soc. Exptl. Biol. Med. 91:143-146, 1956.

7. Leloir, L. F. and Cardini, C. E. Biosynthesis of glycogen from uridine diphosphate glucose. J. Am. Chem. Soc. 79:6340-6341, 1957.

8. Mehrotra, R. M. L. and Agarwal, S. K. Histological and biochemical changes following x-irradiation of exposed liver. J. Pathol. Bacteriol. 91:207-213, 1966.

9. Miquel, J., Klatzo, I., Menzel, D. B. and Haymaker, W. Glycogen changes in X-irradiated rat brain. Acta Neuropathol. 2:482-490, 1963.

10. Miquel, J., Lundgren, P. R. and Jenkins, J. O. Effects of roentgen radiation on glycogen metabolism of the rat brain. Acta Radiol. (Therapy) 5:123-132, 1966.

11. Palade, G. E. A study of fixation for electron microscopy. J. Exptl. Med. 95:285-298, 1952.

12. Rall, T. W., Sutherland, E. W. and Berthet, J. Effect of epinephrine and glucagon on the reactivation of phosphorylase in liver homogenates. J. Biol. Chem. 224:463-475, 1957.

13. Ross, M. H. and Ely, J. O. Radiation effects on liver glycogen in the rat. J. Cellular Comp. Physiol. 37:163-173, 1951.

14. Sjöstrand, F. S. A new ultrastructural element of membranes in mitochondria and of some cytoplasmic membranes. J. Ultrastructure Res. 9:340-361, 1963.

15. Spurlock, B. O., Kattine, V. C. and Freeman, J. A. Technical modifications in Maraglas embedding. J. Cell Biol. 17:203-207, 1963.

16. Streffer, C. Untersuchungen über den Energiestoffwechsel im Lebergewebe der Maus nach Ganzkörperbestrahlung. Int. J. Radiation Biol. 11:179-191, 1966.

17. Sutherland, E. W. and Rall, T. W. The relation of adenosine-3',5'-phosphate and phosphorylase to the actions of catecholamines and other hormones. Pharmacol. Rev. 12:265-299, 1960.

18. Takeuchi, T. Histochemical demonstration of branching enzyme (amylo-1,4 → 1,6-transglucosidase) in animal tissues. J. Histochem. Cytochem. 6:208-216, 1958.

19. Takeuchi, T. and Glenner, G. G. Histochemical demonstration of uridine diphosphate glucose-glycogen transferase in animal tissues. J. Histochem. Cytochem. 9:304-316, 1961.

20. Takeuchi, T. and Kuriaki, H. Histochemical detection of phosphorylase in animal tissues. J. Histochem. Cytochem. 3:153-160, 1955.

21. Venable, J. H. and Coggeshall, R. A simplified lead citrate stain for use in electron microscopy. J. Cell Biol. 25:407-408, 1965.

DISTRIBUTION LIST

AIR FORCE

The Surgeon General, U. S. Department of the Air Force, Washington, D. C. 20333 (1)
Executive Officer, Director of Professional Services, Office of the Surgeon General, Hq. USAF (AFMSPA) T-8, Washington, D. C. 20333 (1)
Headquarters, U. S. Air Force (AFMSPAB), Washington, D. C. 20333 (1)
USAFSAM (SMBR), ATTN: Chief, Radiobiology Branch, Brooks AFB, Texas 78235 (1)
Air Force Weapons Laboratory, ATTN: WLIL (1), ATTN: WLRB-2 (1), Kirtland AFB, New Mexico 87117 (2)
Chief, Nuclear Medicine Department, P. O. Box 5088, USAF Hospital, Wright-Patterson AFB, Ohio 45433 (1)
Office of the Command Surgeon (ADCSG), Hq. ADC, USAF, Ent AFB, Colorado 80912 (1)
Commander, 6571st Aeromedical Research Laboratory, Holloman AFB, New Mexico 88330 (2)

ARMY

The Surgeon General, U. S. Department of the Army, Washington, D. C. 20315 (1)
Surgeon General, ATTN: MEDDH-N, U. S. Department of the Army, Washington, D. C. 20315 (1)
USACDC CSSG, Doctrine Division, Fort Lee, Virginia 23801 (1)
CG, USCONARC, ATTN: ATUTR-TNG (NBC), Fort Monroe, Virginia 23351 (1)
Commanding Officer, U. S. Army Medical Research Laboratory, Fort Knox, Kentucky 40121 (1)
Commanding Officer, USA Nuclear Medical Research Detachment, Europe, APO New York, N. Y. 09180 (2)
Army Research Office, ATTN: Chief, Scientific Analysis Branch, Life Sciences Division, 3045 Columbia Pike, Arlington, Virginia 22204 (1)
Division of Nuclear Medicine, Walter Reed Army Institute of Research, Walter Reed Army Medical Center Washington, D. C. 20012 (5)
Commanding Officer, U. S. Army Environmental Hygiene Agency, ATTN: USAEHA-RP, Edgewood Arsenal, Maryland 21010 (1)
Commandant, U. S. Army Medical Field Service School, ATTN: MEDEW-ZNW, Fort Sam Houston, Texas 78234 (1)

NAVY

Chief, Bureau of Medicine and Surgery, U. S. Navy Department, Washington, D. C. 20390 (1)
Chief, Bureau of Medicine and Surgery, ATTN: Code 71, U. S. Navy Department, Washington, D. C. 20390 (1)
Director, Biological Sciences Division, Office of Naval Research, Washington, D. C. 20360 (1)
Commanding Officer, Naval Aerospace Medical Institute, NAMC, ATTN: Research Director, Pensacola, Florida 32512 (3)
Head, Animal Behavioral Sciences Branch, Naval Aerospace Medical Institute, Naval Aerospace Medical Center, ATTN: Dr. John S. Thach, Jr., Pensacola, Florida 32512 (1)
Commanding Officer, U. S. Naval Hospital, ATTN: Director, REEL, NNMC, Bethesda, Maryland 20014 (1)
Commanding Officer, Nuclear Weapons Training Center, Atlantic, Nuclear Warfare Department, Norfolk, Virginia 23511 (1)
Commanding Officer, Naval Submarine Medical Center, Naval Submarine Base, NL, ATTN: Medical Library, Groton, Connecticut 06340 (1)
Commanding Officer, Naval Submarine Medical Center, Naval Submarine Base, NL, ATTN: Code 53, Nuclear Medicine Training Division, Groton, Connecticut 06340 (1)

D. O. D.

Director, Defense Atomic Support Agency, Washington, D. C. 20305 (1)
Director, Defense Atomic Support Agency, ATTN: DDST, Washington, D. C. 20305 (1)
Director, Defense Atomic Support Agency, ATTN: Chief, Medical Directorate, Washington, D. C. 20305 (4)
Director, Defense Atomic Support Agency, ATTN: Technical Library (APTL), Washington, D. C. 20305 (2)
Commander, Field Command, Defense Atomic Support Agency, ATTN: FC Technical Library, Sandia Base, Albuquerque, New Mexico 87115 (1)
Director, Armed Forces Institute of Pathology, Washington, D. C. 20305 (1)
Administrator, Defense Documentation Center, Cameron Station, Bldg. 5, Alexandria, Virginia 22314 (20)

OTHER GOVERNMENT

U. S. Atomic Energy Commission, Headquarters Library, Reports Section, Mail Station G-17, Washington, D. C. 20545 (1)
U. S. Atomic Energy Commission, Division of Biology and Medicine, Washington, D. C. 20545 (1)
U. S. Atomic Energy Commission, Bethesda Technical Library, 7920 Norfolk Avenue, Bethesda, Maryland 20014 (1)

OTHER GOVERNMENT (continued)

National Aeronautics and Space Administration, ATTN: Lt. Col. Charles M. Barnes, USAF, DB-3, MSC, Houston, Texas 77058 (1)
National Aeronautics and Space Administration, Manned Spacecraft Center, ATTN: Dr. B. D. Newsom, Mail Code DA, Houston, Texas 77058 (1)
National Bureau of Standards, ATTN: Chief, Radiation Physics Division, Washington, D. C. 20234 (1)
U. S. Public Health Service, Bureau of Radiological Health, Division of Biological Effects, 12720 Twinbrook Parkway, Rockville, Maryland 20852 (1)
U. S. Public Health Service, Bureau of Radiological Health, Library, 12720 Twinbrook Parkway, Rockville, Maryland 20852 (1)
U. S. Public Health Service, Northeastern Radiological Health Laboratory, 109 Holton Street, Winchester, Massachusetts 01890 (1)
U. S. Public Health Service, Southeastern Radiological Health Laboratory, P. O. Box 61, Montgomery, Alabama 36101 (1)
U. S. Public Health Service, Southwestern Radiological Health Laboratory, P. O. Box 15027, Las Vegas, Nevada 89114 (1)

OTHER

Argonne National Laboratory, Library Services Department, Report Section Bldg. 203, RM-CE-125, 9700 South Cass Avenue, Argonne, Illinois 60440 (1)
Dr. Donald G. Baker, Radiobiology Department, Zellerbach Saroni Tumor Institute, 1600 Divisadero Street, San Francisco, California 94115 (1)
Dr. J. T. Brennan, Radiology Department, University of Pennsylvania, 3400 Spruce Street, Philadelphia, Pennsylvania 19104 (1)
Brookhaven National Laboratory, Information Division, ATTN: Research Library, Upton, Long Island, New York 11973 (2)
Dr. J. S. Burkle, Director of Nuclear Medicine, York Hospital, York, Pennsylvania 17403 (1)
Director, Radiobiology Laboratory, University of California, Davis, California 95616 (1)
University of California, Lawrence Radiation Laboratory, Library, Bldg. 50, Room 134, Berkeley, California 94720 (1)
University of California, Lawrence Radiation Laboratory, Technical Information Division Library L-3, P. O. Box 808, Livermore, California 94551 (2)
University of California, Laboratory of Nuclear Medicine and Radiation Biology, Library, 900 Veteran Avenue, Los Angeles, California 90024 (1)
Cdr. William H. Chapman, USN (Ret.), Bio-Medical Division L-523, Lawrence Radiation Laboratory, University of California, P. O. Box 808, Livermore, California 94551 (1)
Director, Collaborative Radiological Health Laboratory, Colorado State University, Fort Collins, Colorado 80521 (1)
Dr. L. W. Davis, Radiology Department, University of Pennsylvania, 3400 Spruce Street, Philadelphia, Pennsylvania 19104 (1)
Professor Merril Eisenbud, New York University, Tuxedo, New York 10987 (1)
Dr. T. C. Evans, Radiation Research Laboratory, College of Medicine, University of Iowa, Iowa City, Iowa 52240 (1)
Dr. Arnold Feldman, Institute of Radiology, School of Medicine, Washington University, 510 South Kingshighway, St. Louis, Missouri 63110 (1)
Mr. Orin Gelderloos, Division of Literature, University of Michigan, Dearborn Campus, Dearborn, Michigan 48124 (1)
General Dynamics/Fort Worth, ATTN: Librarian, P. O. Box 748, Fort Worth, Texas 76101 (1)
Gulf General Atomic Incorporated, ATTN: Library, P. O. Box 608, San Diego, California 92112 (1)
IIT Research Institute, ATTN: Document Library, 10 West 35th Street, Chicago, Illinois 60616 (1)
Dr. R. F. Kallman, Department of Radiology, Stanford University, Palo Alto, California 94305 (1)
Dr. L. S. Kelly, Donner Laboratory, University of California at Berkeley, Berkeley, California 94720 (1)
Los Alamos Scientific Laboratory, ATTN: Report Librarian, P. O. Box 1663, Los Alamos, New Mexico 87544 (1)
Director, Nuclear Science Center, Louisiana State University, Baton Rouge, Louisiana 70803 (2)
Lovelace Foundation for Medical Education and Research, Document Library, 5200 Gibson Blvd., S. E., Albuquerque, New Mexico 87108 (1)
Dr. Ross A. McFarland, Guggenheim Professor of Aerospace Health and Safety, Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115 (1)
Dr. J. I. Marcum, Rand Corporation, 1700 Main Street, Santa Monica, California 90401 (1)
Massachusetts Institute of Technology, M.I.T. Libraries, Technical Reports, Room 14 E-210, Cambridge, Massachusetts 02139 (1)

OTHER (continued)

Dr. Charles W. Mays, Physics Group Leader, Radiobiology Division, University of Utah, Salt Lake City, Utah 84112 (1)
Ohio State University, Nuclear Reactor Laboratory, 1298 Kinnear Road, Columbus, Ohio 43212 (1)
Dr. Harvey M. Patt, Laboratory of Radiobiology, University of California, San Francisco Medical Center, San Francisco, California 94122 (1)
Purdue University, Nuclear Engineering Library, Lafayette, Indiana 47907 (1)
Dr. S. M. Reichard, Director, Division of Radiobiology, Medical College of Georgia, Augusta, Georgia 30902 (1)
University of Rochester, Atomic Energy Project Library, P. O. Box 287, Station 3, Rochester, New York 14620 (1)
Dr. H. H. Rossi, 630 West 168th Street, New York, N. Y. 10032 (1)
Dr. Eugene L. Saenger, Director, Radioisotope Laboratory, Cincinnati General Hospital, Cincinnati, Ohio 45229 (1)
Sandia Corporation Library, P. O. Box 5800, Albuquerque, New Mexico 87115 (1)
Scientific Committee on the Effects of Atomic Radiation, ATTN: Library, United Nations Room 3267, United Nations Plaza, New York, N. Y. 10017 (1)
Scope Publications, Franklin Station, P. O. Box 7407, Washington, D. C. 20004 (1)
Dr. Arthur R. Tamplin, Biophysicist, Information Integration Group, University of California, Lawrence Radiation Laboratory, L-612, Livermore, California 94550 (1)
Texas A. and M. University, Radiation Biology Laboratory, Texas Engineering Experiment Station, College Station, Texas 77840 (2)
Texas Nuclear Corporation, ATTN: Director oi Research, Box 9267 Allandale Station, Austin, Texas 78756 (1)
Western Reserve University, Department of Radiology, Division of Radiation Biology, Cleveland, Ohio 44106 (1)
Mr. Lionel Zamore, 601 Brightwater Court, Brooklyn, New York 11235 (1)

FOREIGN

International Atomic Energy Agency, Kärntnerring 11, Vienna I, 1010, Austria (1)
European Atomic Energy Community, C. E. E. A., Library, 51 rue Belliard, Brussels 4, Belgium (1)
Dr. L. G. Lajtha, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, England (1)
Dr. L. F. Lamerton, Biophysics Department, Institute of Cancer Research, Surrey Branch, Belmont, Sutton, Surrey, England (1)
National Lending Library for Science and Technology, Boston Spa, Yorkshire, England (1)
Directorate of Medical and Health Services, FAF (Federal Armed Forces), Bonn, Ermekeilstrasse 27, West Germany (1)
Abteilung für Strahlenbiologie im Institut für Biophysik der Universität Bonn, 53 Bonn-Venusberg, Annaberger Weg 15, Federal Republic of Germany (2)
Prof. Dr. H. Langendorff, Direktor des Radiologischen Instituts der Universität, 78 Freiburg im Breisgau, Albertstrasse 23, Germany (1)
Priv.-Doz. Dr. O. Messerschmidt, Radiologisches Institut der Universität, 78 Freiburg im Breisgau, Albertstrasse 23, Germany (1)
Dr. Helmut Mitschrich, Akademie des Sanitäts- und Gesundheitswesens der Bundeswehr, Spezialstab ATV, 8 München, Schwere Reiterstrasse 4, Germany (2)
Prof. Dr. F. Wachsmann, Gesellschaft für Strahlenforschung m.b.H., 8042 Neuherberg bei München, Institut für Strahlenschutz, Ingolstadter Landstrasse 1, München, Germany (1)
Col. Joachim Emde, Direktor, Spezialstab ATV, ABC- und Selbstschutzschule, 8972 Sonthofen 2/Allgäu, Berghoferstrasse 17, West Germany (1)
Dr. M. Feldman, Section of Cell Biology, The Weizmann Institute of Science, Rehovoth, Israel (1)
Dr. G. W. Barendsen, Radiobiological Institute TNO, Rijswijk, Netherlands (1)
Puerto Rico Nuclear Center, ATTN: Reading Room, College Station, Mayaguez, Puerto Rico 00708 (2)
Dr. H. Cottier, Pathological Institut der Universität, Bern, Switzerland (1)

☆U.S. GOVERNMENT PRINTING OFFICE: 1970-397-177/197

UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Armed Forces Radiobiology Research Institute
Defense Atomic Support Agency
Bethesda, Maryland 20014

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b. GROUP
N/A

3. REPORT TITLE
A HISTOCHEMICAL STUDY OF LIVER ENZYMES INVOLVED IN GLYCOGEN METABOLISM IN THE X IRRADIATED RAT

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (First name, middle initial, last name)
G. N. Catravas and C. G. McHale

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
June 1970	22	21

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. NWER XAXM	AFRRI SR70-6
c. Task and Subtask C 901	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d. Work Unit 01	

10. DISTRIBUTION STATEMENT
This document has been approved for public release and sale; its distribution is unlimited

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Director Defense Atomic Support Agency Washington, D. C. 20305

13. ABSTRACT

The liver enzymes responsible for the breakdown and synthesis of glycogen from glucose have been investigated cytochemically in rats exposed to 1200 rads of x rays. It was found that significant changes occur in their activities and that amylophosphorylase and amylo-1,6-glucosidase, both of which are responsible for the conversion of glycogen to glucose, are markedly inhibited by radiation. A significant inhibition of the activity of 1,4 → 1,6 transglucosidase (branching enzyme) was also observed. In contrast, the activity of UDPG-glycogen transglucosylase, which is responsible for the in vivo synthesis of 1,4-polysaccharides, was found to be enhanced.

DD FORM 1 NOV 65 1473 (PAGE 1)
S/N 0101-807-6801

UNCLASSIFIED
Security Classification